IOWA STATE UNIVERSITY
Digital Press

Animal Industry Report

Animal Industry Report

AS 664

ASL R3213

2018

Observations Regarding Depressed Cholinesterase in Beef Cattle after Feeding Contaminated Corn Silage

Garland Dahlke
Iowa State University, garland@iastate.edu

Observations Regarding Depressed Cholinesterase in Beef Cattle after Feeding Contaminated Corn Silage

A.S. Leaflet R3213

Garland Dahlke, Extension Program Specialist,
Iowa Beef Center

Summary and Implications

Beef cattle fed corn silage with an unidentified mycotoxin or possibly some other metabolite expressed a great decline in blood and brain cholinesterase activity. The presence of the ionophore in the ration appeared to make the depression much more severe initially and although blood cholinesterase levels did rebound somewhat after a period of time, brain cholinesterase levels did not recover to what would be considered normal even though the ionophore was removed from the ration.

Introduction

Cattle on a large eastern Iowa farm which ran both a beef feedlot and cow-calf operation were plagued with a higher than usual mortality rate. The problem was first noticed with 5 dead pregnant cows at one location, followed by a large number of pregnant cows losing their pregnancy at various stages of parturition during the winter while the cows were being fed stored feed. These problems began in late November and continued throughout the winter and into the following summer. Calves that were carried to term tended to appear normal but were extremely lethargic at birth and often would not nurse or even attempt to nurse on their own. Necropsies were performed on calves and on cows that died. The animals appeared to be fine however caudate nucleus of the brain showed highly depressed cholinesterase activity (below 0.4 delta pH units/hour). Likewise, the cattle in the feedlot of this farm also displayed the depressed cholinesterase activity in the caudate nucleus (observed in those that died) and frequently in the blood of those appearing sick. The mortality rate in the feedlot cattle did not seem to be greatly different than what was observed in previous years, but was higher. Closeout performance on finished cattle may have been slightly poorer than normal, but this aspect was not obvious. Table 1 provides a summary of the cattle that were tested over this period of time. Blood was used on animals that appeared sick, but still alive, while brain tissue was used from animals that had died.

Due to the symptoms it was first thought that the cattle were exposed to an organophosphate or carbamate insecticide. Feed, water and soil samples were submitted to the Iowa State and the Michigan State Universities veterinary diagnostic laboratories along with Brookside Laboratory of New Bremen, Ohio. None of these compounds were discovered.

Table 1. Summary of Number of Animals Tested for Cholinesterase Activity Prior to Trial

	Blood	Brain
Total Samples	197	118
Low Activity (below 0.40 delta pH units/hour)	21	99
Marginal Activity (0.4 – 0.45 delta pH units/hour)	13	3
Adequate Activity (above 0.45 delta pH units/hour)	162	16

Material and Methods

Three tasks were addressed. First was to confirm the cause of the problem. Second was to determine some means of managing the issue on a practical basis and finally to see if there were any long-term effects from exposure.

Feedstuffs were systematically excluded from rations of new cattle as they entered the feed yard and feed samples were taken from all the ingredients. These samples were mailed to Dairyland Laboratories of Arcadia WI to evaluate mold and yeast counts along with the lab's mycotoxin panel that covered aflatoxin, zearalenone, T2 toxin and vomitoxin (DON). Testing was also done with two other laboratories; Altech ™ using their commercial panel of 37 mycotoxins and the 400-compound panel ran by Quantas Analytics ™ at University of Tulln in Austria.

The corn silage being suspect, new cattle were then purchased from sale barns in South Dakota. Since the actual compound causing the problem was not known and the quantity of feed massive, a trial to look at a means of both managing the problem as well as determining if exposure would lead to any long-term problems was initiated. The cattle purchased were mature, cull cows and these would be now fed for the "white fat" beef market. Cows were split into two groups as unloaded from trucks, checked for pregnancy, weighed and a blood sample was taken.

Pregnant cows were removed from the groups and the blood samples were sent to the Iowa State University Diagnostic Laboratory to evaluate cholinesterase levels. The two groups of cows were then provided a ration of the suspect corn silage and soybean meal. Since ionophores fed to ruminants tend to reduce protozoa in the rumen and being that protozoa show some efficacy in metabolizing mycotoxins one group (Gi) was provided an ionophore (Cattlyst ®) in the ration at 125 mg per head daily while the other (Gc) was not. Blood samples and weights were gathered at three weeks and six weeks into the feeding period. Cattle in these two groups were fed for a total of 140 days. At this time the cattle were to be processed and the brain caudate nucleus harvested and evaluated for cholinesterase activity at the Iowa State University Veterinary Diagnostic Laboratory.

Results and Discussion

The mold and yeast counts in the corn silage were higher than any of the others. This may have been a tip in finding the causative agent, but all of the ingredients were tried considered as possible agents in leading to the condition. Of the ingredients tested only the corn silage seemed to have impact on cholinesterase activity when included in the rations. With the mycotoxin laboratory analysis, none of the individual mycotoxins tested in the Dairyland Laboratory were considered beyond what would be a safe level in cattle rations (see Appendix A). Data from the Alltech37+ ® report (see Appendix B) indicated an extremely high level of Type B Trichothecenes (which would include deoxynlvalenol) but no other of consequence. Data from the Quantas Analytics ™ (see Appendix C) also indicates the same trichothecenes along with a number of other compounds, but none are mentioned to be strong cholinesterase inhibitors in the literature.

Initial blood cholinesterase activity and weight data are provided in Table 2 from the cull cows Gi and Gc. A "Z" statistic was used to test whether there was a difference between the groups Gi and Gc. There was no substantial difference between groups Gi and Gc with initial blood cholinesterase. However, by week three there was a significant difference in blood cholinesterase measures between groups Gi and Gc (*P(Z<=z) = 0.0007). Likewise, the drop from the initial 0.68 delta pH/hour to 0.44 delta pH/hour in the Gi group was significant *P(T<=t) = 0.0002). The drop in cholinesterase activity in group Gc individuals from the initial measure to day 21 was not great.

The Gi group turned out to be considerably lower in off truck weight than Gc P(Z<=z) = 0.04). This was just a function of the initial gate sort as cattle entered the feedlot which simply alternated putting cattle in one group or the other. Weight gain during these first three weeks did have the effects of gut fill. This gain although quite large in numerical difference was quite variable between cows in the groups and did not register as significant at this point from a statistical view.

Table 2. Initial Weights and Blood Cholinesterase through Week Three

	N	Day 0 Blood Cholinesterase*	Day 21 Blood Cholinesterase	P(T<=t)		Day 0 Weight	Day 21 ADG
Gi	20	0.68	0.44	0.0002		1257	4.80
Gc	15	0.63	0.57	0.10		1324	6.78
P(Z<=z)		0.24	0.0007			0.04	0.11

*Units are delta pH/hr

**The nomenclature of P(Z<=z) or P(T<=t) indicates that with a null hypothesis that assumes the difference between the sample and the population is zero, the probability (P) of the treatment sample (z or t) difference from the population (Z or T) is still zero is given. The smaller the value, the less likely the null hypothesis holds true, thus there is a treatment effect.*

The Z statistic was used to compare between groups Gi and Gc. The T statistic was used to compare within each group.

After six weeks blood cholinesterase levels and weight gain rebounded in group Gi (see Table 3). The visible appearance of the Gi cattle at this point however was considerably worse than those of the Gc group and caused anxiety in the cooperating feedlot. The management, not willing to risk a greater financial loss decided to remove the ionophore from Gi. At this time, until the end of the trial the ration used between groups was the same. From here, the study was to assess long term problems looking at the brain cholinesterase activity in the caudate nucleus at day 140 when cattle were harvested.

Table 3. Initial Weights and Blood Cholinesterase through Week Six

	N	Day 0 Blood Cholinesterase	Day 42 Blood Cholinesterase	P(T<=t)		Day 42 Weight	Day 42 ADG
Gi	20	0.68	0.60	0.09		1432	4.16
Gc	15	0.63	0.60	0.22		1493	4.02
P(Z<=z)		0.24	0.43			0.07	0.42

Although the blood cholinesterase levels recovered after week six, it does appear that the compound contaminating this corn silage is quite potent and has long term effects in the brain. Table 4 provides a summary of the initial blood versus the final brain cholinesterase activity. Both groups showed a significant decrease from the initial blood cholinesterase activity levels assuming the initial blood and brain activities were similar. The brain levels did not seem to rebound as the blood levels did if this assumption is correct. The brain cholinesterase activity in group Gi was not considered significantly different than group Gc at day 140 although Gi was considered below normal at this point while group Gc brain cholinesterase activity was at the bottom of the normal range (normal is considered 0.40 or greater delta pH/hr).

Table 4. Initial Weights and Blood/Brain Cholinesterase through Week Twenty

	N	Day 0 Blood Cholinesterase	Day 140 Brain Cholinesterase	P(T<=t)		Day 140 Weight	Day 140 ADG	Final CarcassWt
Gi	20	0.68	0.35	0.00005		1671	2.96	925
Gc	15	0.63	0.40	0.0006		1709	2.75	953
P(Z<=z)		0.24	0.28			0.19	0.23	0.17

In retrospect when considering the rest of the cattle on the farm, this may explain some of the sampling anomalies that were observed in the herd up to the point of this trial. For instance, a number of newborn calves that died shortly after birth and delivered three months after their dams consumed this silage still had highly impaired brain cholinesterase levels while the dams of these calves exhibited blood cholinesterase activity in a normal range. The effect in the brain may even be wider reaching if one considers the compromised reproduction rates observed with the replacement heifers in the breeding season that followed their feeding of this corn silage as yearlings where only about 30% of these females conceived and many never exhibited any estrous cycle.

Toxins produced by molds like *Stachybotrys chartarum* have been shown to lead to long term impaired cholinesterase activity in humans, but the causative agent has not been identified in this feed. A sample of this feed was taken from the bunker silo and a mold identification panel was performed on the sample by the Iowa State University Diagnostic Laboratory. The panel revealed high levels of *Aspergillus fumingatus*, and *Zygomycetes* species and a trace of *Trichosporon* species. It is quite probable that these organisms however set in after harvest and were not contributors to the problem since these organisms tend to be ubiquitous and common in decaying organic matter This is unfortunate since the remedy could not be identified either. A binding agent such as a clay or activated charcoal may have some merit as may an enzyme or other metabolizing agents. It does appear that the normal benefits of providing an ionophore in the ration are lost in situations where the feed is contaminated. Thus it may be best to refrain from feeding the ionophore with mycotoxin

contamination, since the natural rumen microflora appear to be the best-known defense.

As a follow up, using the new crop silage, a new group of cattle were tested for blood cholinesterase activity at placement in the feedyard and then three weeks later after feeding this new crop silage (Table 5). Here, also, the ionophore was included in one group and not included in the other. Both groups maintained cholinesterase activity in the normal range although the group receiving the ionophore did show a significant reduction in activity from their initial values. Weight gain was not strongly different between groups at this three week point. After five months of storage the new crop silage has yet to produce a visible problem while the contaminated silage caused a noticeable problem throughout the time cattle were provided that silage. With this in mind, it seems that the issue may have risen from the growing crop in the field rather than a problem in storage. The effect of an ionophore in the ration also should not be ignored since it seems to make the condition worse.

Table 5. Retest of Cholinesterase Activity with New Crop Corn Silage and New Cattle

	N.	Day 0 Blood Cholinesterase	Day 21 Blood Cholinesterase	P(T<=t)		Day 21 ADG
newGi	20	0.59	0.45	0.00001		2.29
newGc	19	.065	0.61	0.08		2.87
P(Z<=z)		0.06	3.4×10^{-10}			0.47

Appendix A – Dairyland Laboratory Report

DESCRIPTION: 2015 corn silage

DATE	2/8/16	2/23/16
RESULTS:	AS IS	AS IS
MOLD COUNT		<1,000 cfm/gm
YEAST COUNT		22,900,000 cfm/gm
MOISTURE	58.69%	59.26%
DRY MATTER	41.31%	40.74%
	DRY BASIS	DRY BASIS
AFLATOXIN	9.4 ppb	none detected
ZEARALENONE	none detected	none detected
VOMITOXIN	1.1 ppm	0.7 ppm
T-2 TOXIN	none detected	none detected

Appendix B – Alltech 37+ ® Report

Mycotoxin Analysis Report

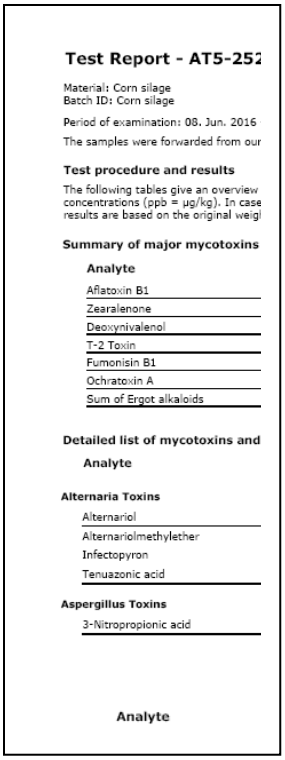
Alltech 37+® RESULTS: MYCOTOXINS LEVELS MEASURED AT 94.41 % DRY MATTER

Sample ID #:	IA3076	Customer Sample ID:	Corn silage
Origin:	Anamosa, IA	Feed Matrix:	Corn silage
Species:	Beef		

Internal Ref # 115-012-11611	Mycotoxins	Levels Detected (ppb)	± Stdev (ppb)	Detection Limit (ppb)	Lower Quantification Limit (ppb)
1	Aflatoxin B1	ND	ND	0.129	0.429
2	Aflatoxin B2	ND	ND	0.684	2.281
3	Aflatoxin G1	ND	ND	0.449	1.495
4	Aflatoxin G2	7.01	3.88	0.422	1.408
5	Ochratoxin A	ND	ND	0.362	1.208
6	Ochratoxin B	ND	ND	0.302	1.008
7	Deoxynivalenol	6365.51	714.18	5.713	19.044
8	3-AcDon	ND	ND	4.058	13.526
9	15-AcDon	ND	ND	7.442	24.806
10	DON-3-Glucoside	ND	ND	16.651	55.500
11	Nivalenol	ND	ND	53.988	179.960
12	Fusarenon X	ND	ND	2.489	8.295
13	Fusaric Acid	631.94	67.33	0.017	0.055
14	T2 Toxin	ND	ND	0.744	2.481
15	HT2 Toxin	ND	ND	2.296	7.655
16	Diacetoxyscirpenol	ND	ND	1.505	5.017
17	Neosolaniol	ND	ND	0.946	3.154
18	Fumonisin B1	ND	ND	20.426	68.086
19	Fumonisin B2	ND	ND	1.804	6.012
20	Fumonisin B3	ND	ND	2.918	16.493
21	Zearalenone	ND	ND	2.545	8.482
22	α Zearalanol	ND	ND	12.964	43.213
23	β-Zearalanol	ND	ND	10.910	36.467
24	Zearalanone	ND	ND	3.427	11.424
25	Patulin	ND	ND	16.669	55.562
26	Mycophenolic Acid	ND	ND	2.496	8.319
27	Roquefortine C	1.32	0.54	0.196	0.653
28	Penicillic Acid	ND	ND	11.693	38.978
29	Wortmannin	ND	ND	0.764	2.545
30	Gliotoxin	ND	ND	5.608	18.692
31	Sterigmatocystin	ND	ND	0.184	0.612
32	Verruculogen	ND	ND	0.331	1.104
33	2-Bromo-Alpha-Ergocryptine	ND	ND	0.838	2.794
34	Ergometrine/Ergonovine	ND	ND	0.573	1.911
35	Ergotamine	ND	ND	0.502	1.673
36	Lysergol	ND	ND	0.457	1.522
37	Methylergonovine	ND	ND	0.048	0.161
38	Alternariol	ND	ND	1.379	4.598

ND: Not Detected, value is below the limit of quantification.

Appendix C - Quantas Analytics ™ Report



Test Report - AT5-252

Material: Corn silage
Batch ID: Corn silage

Period of examination: 08. Jun. 2016

The samples were forwarded from our

Test procedure and results

The following tables give an overview
concentrations (ppb = µg/kg). In case
results are based on the original weigl

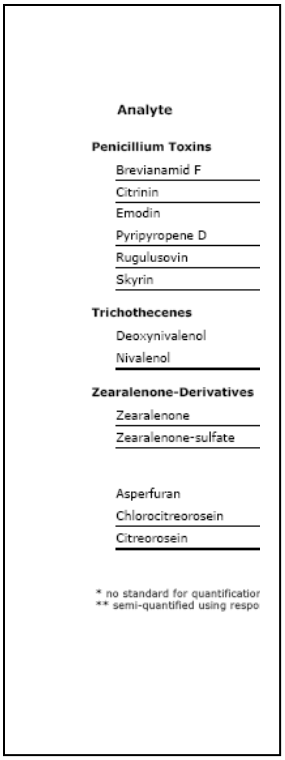
Summary of major mycotoxins

Analyte
Aflatoxin B1
Zearalenone
Deoxynivalenol
T-2 Toxin
Fumonisin B1
Ochratoxin A
Sum of Ergot alkaloids

Detailed list of mycotoxins and

Analyte
Alternaria Toxins
Alternariol
Alternariolmethylether
Infectopyron
Tenuazonic acid
Aspergillus Toxins
3-Nitropropionic acid

Analyte



Analyte
Penicillium Toxins
Brevianamid F
Citrinin
Emodin
Pyripyropene D
Rugulusovin
Skyrin
Trichothecenes
Deoxynivalenol
Nivalenol
Zearalenone-Derivatives
Zearalenone
Zearalenone-sulfate
Asperfuran
Chlorocitreorosein
Citreorosein

* no standard for quantificatior
** semi-quantified using respo